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Simultaneous determination of diphenylamine and nitrosodiphenylamine by photochemically induced fluorescence and synchronous fluorimetry using double scans method

Naader Alizadeh *, Alireza Farokhcheh

Department of Chemistry, Faculty of Sciences, Tarbiat Modares University, PO Box 14115-175, Tehran, Iran

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ABSTRACT

A new synchronous fluorimetry in the combination with the photochemically induced fluorescence (PIF) method for simultaneous determination of nitrosodiphenylamine (NDPhA) and diphenylamine (DPhA) in aqueous and methanolic solution has been proposed. DPhA has a variety of applications and NDPhA is carcinogenic product of its. The method is based on the use of UV irradiation to produce fluorescent derivatives from NDPhA as a non-fluorescent molecule. The PIF properties of these amines in three media (water, methanol and acetonitrile) are reported. Because of their similar features, the fluorescence emission spectra of NDPhA photoproducts and DPhA were found to severely overlap in the whole wavelength region. Overlapping of conventional fluorescence spectra of these molecules is resolved by synchronous fluorometry using double scans method (SF-DS), thus making the use of separation techniques unnecessary for simultaneous determination of NDPhA and DPhA. The synchronous fluorescence intensity of NDPhA was measured at $\Delta\lambda$ of 127 nm and at $\Delta\lambda$=75 nm for DPhA in solution, which are independent of each other. The best sensitivity can be achieved in water. The linear ranges for determination of NDPhA and DPhA were 1×10^{-8} to 6×10^{-6} mol L^{-1} and 4×10^{-8} to 9×10^{-6} mol L^{-1}, and the limits of detection (LOD) were 8×10^{-9} and 1×10^{-8} mol L^{-1}, respectively. The relative standard deviations (R.S.D.) of method is < 3% (n=5). The proposed method was successfully applied for the determination of the two compounds in synthetic solutions, well water samples and also in gunpowder samples. The results obtained were favorably compared to those obtained with HPLC analysis.

1. Introduction

Diphenylamine (DPhA) is widely used as an insecticide, a storage preservative for apples, a rubber antioxidant, and a solid fuel rocket propellant, and it has been detected as an environmental pollutant in surface water and groundwater [1]. Nitrosamines, as a group of emerging disinfection byproduct (DBP) [2], have recently caused significant concerns among drinking water utilities because these compounds are generally much more potent rodent carcinogens than the currently regulated DBPs, and because most of nitrosamines are classified as probable human carcinogens by the International Agency for Research on Cancer [3,4]. NDPhA is also considered a probable human carcinogen and one of the five nitrosamine DBPs included in the Contaminant Candidate List 3 [5]. The U.S. EPA Integrated Risk Information Service (IRIS) lists a maximum concentration of 7 μg L^{-1} in drinking water for NDPhA based on a lifetime cancer risk of 1 in 1000,000 [6]. Boyd et al. [7] found that in vitro NDPhA (IC50: 0.6–1.9 × 10^{-3} mol L^{-1}) was substantially more cytotoxic than *N*-nitrosodimethylamine (IC50: 15–95 × 10^{-3} mol L^{-1}) in one animal and three human cell lines. Limited in vivo testing showed that NDPhA could cause bladder cancer in rats [8]. Zhou et al. [9] reported that DPhA as a key precursor of NDPhA, and described the effect of water pH and chloramination conditions on the formation of NDPhA.

Smokeless gunpowders and primary propellant, nitrocellulose (NC), degrade slowly with time and especially under hot conditions. Likewise if nothing is done to stop this degradation an auto-ignition can occur which leads to serious damage [10]. DPhA is the most commonly used stabilizer to control this slow thermal degradation. Typical bulk gunpowders contain from 0.51 to 0.89% DPhA, which limits nitrocellulose decomposition arising from exposure to the acid products of that decomposition [1,11]. During the decomposition reactions, the original stabilizer is consumed and a number of derivatives, such as NDPhA and nitro derivatives of the DPhA are formed [12,13]. DPhA and the carcinogenic NDPhA cannot be directly distinguished by gas chromatography (GC) or GC/mass spectrometry (MS) because NDPhA decomposes at relatively low injection port or

* Corresponding author. Tel.: +98 21 82 88 3409; fax: +98 21 82 88 3455.
E-mail address: alizaden@modares.ac.ir (N. Alizadeh).

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column temperatures to produce DPhA [14]. Therefore, the GC retention times and mass spectra observed for the two compounds are identical. One approach to the determination of both compounds in a sample is their separation prior to GC and other approach is develop a method that can separate DPhA and NDPhA without decomposition and unequivocally identify and measure them such as high-performance liquid chromatography(HPLC) or thermospray HPLC/MS [15,16].

Identification of those compounds has become of great importance in industrial chemical operations; they are routinely included in lists of target compounds sought during investigations of hazardous waste sites. Additionally, determination of DPhA and their derivatives in variety of fields such as pesticides and agriculture, pharmaceutical, rubber, plastics is of high importance.

The techniques which have been most frequently used to determine different stabilizers are as follows: high-performance liquid chromatography with electrochemical detection (LCEC) [17] and with mass spectrometry detection [18], ion mobility spectrometry [19], TLC and LC [20], LC-tandem MS method [21] and fluorescence method [22]. Although, fluorescence-based methods are highly sensitive and simple as compared to the chromatographic methods; but many of the organic compounds present as stabilizer or derivatives are, in general, non fluorescent. For this reason, a number of analysis methods with derivatization process have been developed to convert non-fluorescent stabilizer into highly fluorescent derivatives for the purposes of detection. However, derivative techniques are the most challenging and time-consuming step in the analytical process. On the other hand, problems with interferences in complex matrices can occur during analysis. As a result, using of fluorimetric method for determination of stabilizer has received less attention.

Synchronous fluorescence spectrometry [23] is a simple modification of the conventional fluorescence technique affording higher selectivity thanks to the narrowing of spectral bands and the simplification of spectra [24]. This technique could not only maintain the sensitivity, but also simplify the emission spectra, improve the selectivity and spectral resolution, decrease the interference due to light scattering. These features make this technique suitable for use in the resolution of mixtures involving spectral overlap problems. The maximum fluorescence intensity obtained with synchronous scanning appears when the wavelength increment corresponds to the difference between the wavelengths of excitation and emission maxima and coincides with the maximum signal obtained in the emission spectrum of conventional scan. Synchronous fluorescence is known as a useful technique to carry out simultaneous determinations of multicomponent samples without any pre-treatment [25–27].

In last year's, there has been demonstrated that fluorescence emission of some non- or weakly fluorescent pesticides or photoreactive drugs can be photochemically induced by UV irradiation. The PIF methods are based on the direct UV irradiation of stationary liquid solutions or dynamic flow containing a non fluorescent or weakly fluorescent analyte and photoconversion into strongly fluorescent photoproducts. Generally, photochemical reactions are relatively rapid and their use for the determination of a lot of compounds offers advantages such as simplicity, high sensitivity, high selectivity and cleanliness over other derivatization methods. Also this method can be coupled with chromatographic methods [28–30]. N1-substituted diphenylamines containing an electron deficient species are weakly or non-fluorescent and their direct spectrofluorimetric determination is not feasible, also photochemical decomposition of different diphenylamine derivatives has been reported previously [31].

In this work, we have developed a PIF method for simultaneous determination of NDPhA and DPhA by synchronous fluorometry in different samples. To our best knowledge, no fluorimetric method has been published for simultaneous determination of NDPhA and DPhA by using synchronous fluorometry with double scans method. After investigation of the effect of various physicochemical parameters, including solvent system, oxygen dependence and UV irradiation time, a combination of PIF method and synchronous fluorometry with double scans was carried out for the analysis of these compounds.

2. Materials and methods

2.1. Materials

Diphenylamine, *N*-nitrosodiphenylamine, methanol and acetonitrile were purchased from Sigma-Aldrich. All used reagents were of analytical grade. Double-distilled water was used throughout. Stock solutions of NDPhA and DPhA at the concentration of 0.01 mol L^{-1} were prepared in methanol and kept in the dark at 4 °C for no longer than 3 weeks. Working concentrations of NDPhA and DPhA were prepared from the stock solutions by appropriate dilution before use. Before fluorescence measurements, the working solutions were analyzed by recording UV–visible spectrum from 200 to 800 nm to minimize the inner filter effects effects (fluorescence is uniformly distributed in absorbance < 0.01). All samples prepared from gunpowders were filtered through a Whatman no. 1 filter before further use.

2.2. Apparatus

All PIF measurements were performed at room temperature on a Perkin-Elmer model LS 50B spectrofluorimeter equipped with a thermostated cell compartment. The NDPhA and DPhA fluorescence intensity were measured at the maximum emission wavelength of 363 and 360 nm after excitation of solutions at 233 and 285 nm, respectively. The excitation and emission slits were both maintained at 10 nm. The scan rate of the monochromators was maintained at 40 nm min^{-1} in recording conventional spectra, and at 100 nm min^{-1} for the acquisition of three-dimensional excitation–emission spectra. All measurements were performed in 10 mm quartz cells, at 25 ± 0.1 °C, by use of a thermostatic cell holder and a Thermomix thermostatic bath. Absorption spectra were obtained using a Sinco (model UVS-2100) UV–vis spectrophotometer. The photochemical set-up included a light-box consisting of a mercury lamp (8 W). A standard Hellma (Mullheim, Germany) 1 cm pathlength quartz fluorescence cuvette was placed at 30 cm from the lamp.

2.3. Sample preparation

Stock solutions of stabilizers (10^{-2} mol L^{-1}) were freshly prepared by dissolving the compound in methanol. Serial dilutions were performed to obtain working standard solutions. All solutions were protected against light with aluminium foil and stored in a refrigerator. Before fluorimetry, mixture of NDPhA and DPhA solution was placed in a quartz cuvette and irradiated for a fixed time at room temperature.

2.4. Calibration curves for synchronous fluorimetry

Standard solutions of NDPhA and DPhA (in the working concentration range) were transferred into a two separate series of 20 mL volumetric flasks and were diluted to the volume with solvent. Synchronous spectra were obtained by scanning both monochromators simultaneously at constant wavelength differences of $\Delta\lambda = 127$ nm and $\Delta\lambda = 75$ nm for NDPhA and DPhA, respectively. The synchronous fluorescence intensity measurements were made at the synchronous maxima of 233 and 285 nm for NDPhA and DPhA, respectively.

2.4.1. Gunpowder sample preparation by ultrasonic solvent extraction

Five different gunpowders were analyzed. The compositions of the different gun powders were unknown to us. In order to prepare each gunpowder sample, amount of 50 mg grinded gunpowder was placed in a 25 mL beaker, and then samples were extracted three times with 10 mL of methanol. Extraction time was 10 min and the mixture was agitated in the ultrasonic bath for in each step. Extracts were filtrated immediately after each extraction step through Whatman no. 1 filters before further use. The prepared samples were kept in the dark before analysis [16].

2.5. Chromatography

The liquid chromatographic analysis was performed on a HPLC system with UV PDA detector, model Smartline 2600 (Knauer, Germany). A C18 column 4.6 i.d. × 250 mm, 5.0 mm (Knauer, Germany) was used for separations. The mobile phase was acetonitrile/water (67:33 v/v) and operated at 0.9 mL min^{-1}. The absorption detector was fixed at 283 nm and the peak area of 5 mL sample injection volume was used as the quantification parameter [32].

3. Results and discussion

3.1. Photochemically induced fluorescence behavior

The effect of UV irradiation on fluorescence emission of NDPhA and DPhA in different solvents was studied. Fig. 1 shows typically the excitation and emission spectra of NDPhA and DPhA before and after UV irradiation in water. It is obvious that DPhA has an excitation peak at 285 nm and shows intrinsic fluorescence maximum located at 360 nm, when excited by radiation. Moreover, no significant changes were found in either intensity or maxima wavelength of DPhA after UV irradiation. On the other hand, when NDPhA was irradiated by UV, a maximum in emission spectrum was appear (in 363 nm) with the excitation wavelength in 233 nm. Fluorescence signals of NDPhA (as a naturally non-fluorescent molecule) created upon UV irradiation implicate the formation of one or more emissive photoproducts.

Encinas et al. shown that the chlorodiphenylamin derivatives can undergo photocyclization and yield stable photoproducts under UV irradiation [31,33]. Also, there exist several reports for homolysis of the nitrogen–nitrogen bond. The three main photoproducts when a solution of NDPhA is irradiated by UV radiation were identified as: 9H-carbazole (II), 4-nitrosodiphenylamine (III) and 1,1,2,2-tetraphenyl hydrazine (IV) (Fig. 2), which the priority of products, based on reaction yielding is: IV > III > II [34]. However, more experiments are necessary to obtain the structure of fluorescent photoproduct(s).

The results showed that the kinetics of photodegradation of NDPhA is solvent dependent (faster in water relative to the methanol and acetonitrile). As shown in Fig. 3, the fluorescence increases with the irradiation time till reach a maximum value corresponding to the optimum irradiation time, and then the intensity become constant for a while and finally a slight decrease in intensity is appeared. The shape of the curve obtained suggests a two-step photolysis mechanism, consisting of the formation of a strongly fluorescent photoproduct and the posterior photodegradation of the latter compound into non-fluorescent product(s). Since fluorescence signal obtained a maximum at 5 and 30 min for water and methanol, respectively. So, these values were selected as optimal. Stability of the fluorescent photoproduct was tested and it was found that no remarkable changes occur in fluorescence intensity for more than 6 h (in the absence of UV irradiation).

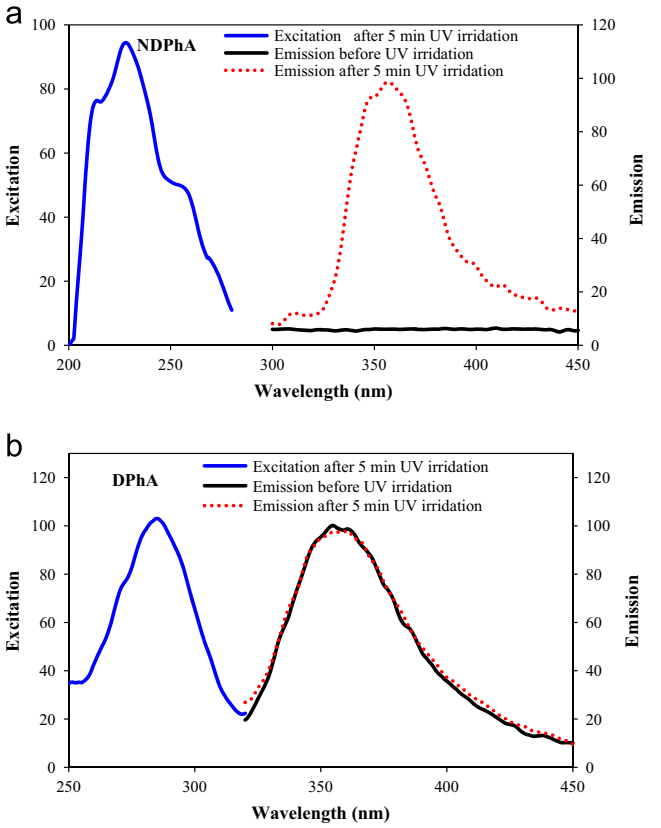

Fig. 1. Fluorescence emission (right axis) and excitation (left axis) spectra of (a): NDPhA (3×10^{-8} mol L^{-1}) and (b): DPhA (9×10^{-8} mol L^{-1}) before (solid line) and after (dash line) UV irradiation in water.

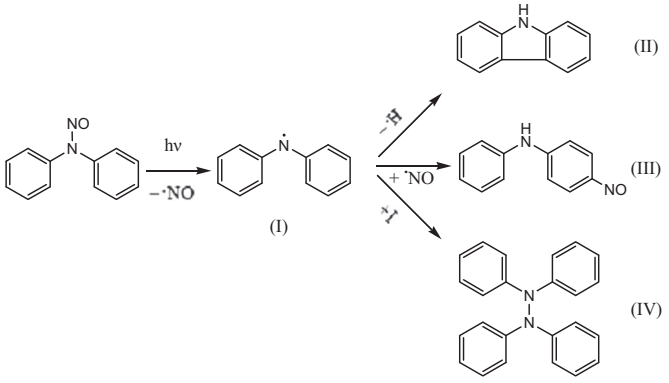

Fig. 2. Possible mechanistic pathways for the photochemical reaction of NDPhA.

3.2. Solvent and oxygen dependence of the PIF

As it is described solvent can change both the extension of the fluorescence intensity and the photo reaction [35] hence, photochemical behavior of NDPhA was evaluated using three different solvents; acetonitrile (aprotic solvent), water and methanol (as protic solvents). As shown in Fig. 3, compared with methanol and acetonitrile, water as a solvent produce a larger increase of fluorescence intensity, also its maximum emission appeared in shorter irradiating time (5 min). The fluorescence excitation and emission spectra of NDPhA photoproduct(s) are practically independent of the polarity of the solvent and no significant shift of the excitation and emission wavelengths occurred on changing the solvent. In these medium, excitation and emission wavelengths of 233 and 363 nm, respectively, were found for photoproduct of NDPhA.

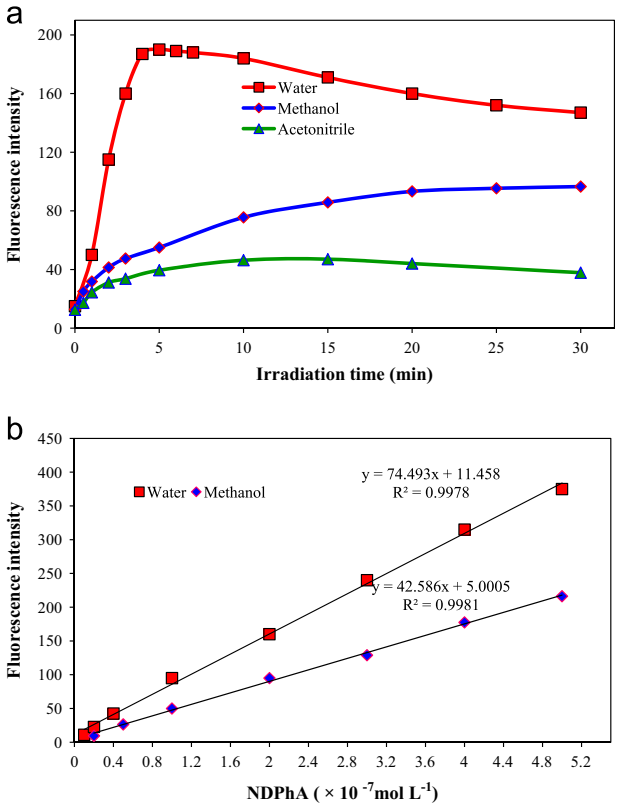

Fig. 3. Effect of irradiation time and solvent type on the PIF intensity of 1×10^{-7} mol L^{-1} NDPhA (a) and calibration sensitivity (5 min UV irradiation for water and 30 min irradiation for methanolic solution) (b); Solvent: water (■), methanol (◆) and acetonitrile (▲).

Water and methanol can contain up to 2.6×10^{-4} mol L^{-1} 1.2×10^{-3} mol L^{-1} of dissolved oxygen at 25 °C, respectively, an amount that can not only alter fluorescence intensity, but also change the PFI mechanisms and product(s). To investigate the possibility of above mentioned effect of O_2, the aqueous and methanolic solution of NDPhA was purged with N_2 in a septum-sealed cell for 20 min and then was irradiated with UV radiation (5 min for water and 30 min for methanol). Our results show that negligible difference in the fluorescence intensity of two solutions of purged and unpurged NDPhA solution with same concentration is obtained.

3.3. Fluorescence excitation and emission spectra of NDPhA and DPhA

The excitation and emission spectra of NDPhA and DPhA are shown in Fig. 1. NDPhA shows an excitation maximum at 233 nm (after UV irradiation), and DPhA shows an excitation maximum at 285 nm. The emission spectra show maxima at 363 and 360 nm for NDPhA and DPhA, respectively. Obviously, the emission spectra of NDPhA and DPhA were overlapped seriously (Fig. 4). As a result, simultaneous analysis of the mixture would not be feasible by conventional spectrofluorometry at their wavelength maxima.

The extent of the overlap of these compounds was examined by obtaining the total spectrofluorimetric information available in the three-dimensional spectra (Fig. 5a) and the excitation–emission matrix (Fig. 5b) of NDPhA (3×10^{-8} mol L^{-1}) and DPhA (9×10^{-8} mol L^{-1}) in water, where the emission spectra at constant increments of the excitation wavelength have been recorded and plotted.

The synchronous fluorescence spectra of NDPhA and DPhA in their mixture were shown in Fig. 5c, which were obtained at

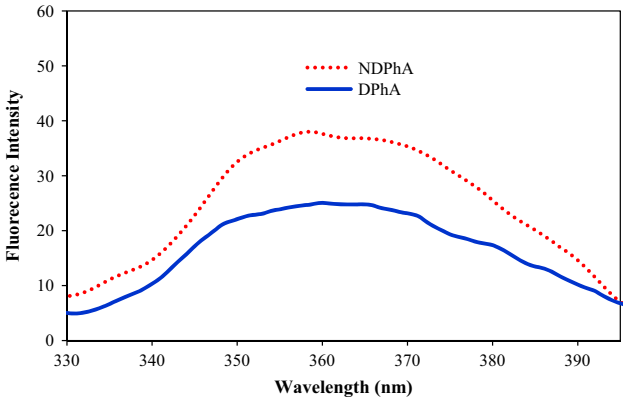

Fig. 4. Conventional fluorescence emission spectra of NDPhA, dash line (3×10^{-8} mol L^{-1}) and DPhA, solid line (5×10^{-8} mol L^{-1}).

a constant interval between the emission and excitation wavelength in double scan mode. Synchronous fluorescence spectra of NDPhA were obtained by simultaneously scanning the excitation and emission monochromators in the excitation wavelength range 210–350 nm, with constant wavelength differences between the emission and excitation wavelengths $\Delta\lambda=\lambda_{em}-\lambda_{ex}=130$ nm. Also, synchronous fluorescence spectra of DPhA were obtained by maintaining a constant interval ($\Delta\lambda=75$ nm) between emission and excitation wavelength at 210 and 350 nm. Fig. 5c shows that, the maximum fluorescence intensity was observed at excitation wavelength 233 nm for NDPhA and at 285 nm for DPhA. When synchronous double scan technique was applied, for the binary mixture NDPhA and DPhA, using a 130 nm value for NDPhA, only one single synchronous band at 363 nm was obtained, because the interval 130 can be found to match solely one pair of excitation and emission bands. Similarly, at $\Delta\lambda=75$ nm, only DPhA yields a detectable signal that is independent of the presence of NDPhA (Fig. 5c). As shown, the peaks corresponding to NDPhA and DPhA were well resolved and the maximum fluorescent signals were at 233 and 285 nm for NDPhA and DPhA, respectively, so it is possible to determine these two molecules simultaneously from a mixture by synchronous fluorescence spectra with double scan technique.

3.4. Contour plots for selection of optimum $\Delta\lambda$

The optimum $\Delta\lambda$ value is an essential factor for performing the synchronous fluorescence scanning technique with regards to its resolution, sensitivity and features. The ordinary way of determining the best $\Delta\lambda$ value for the resolution of a mixture by synchronous spectrofluorimetry involves obtaining the contour plots of the each of the samples alone and the mixture of them.

Fig. 6 shows the contour plot of total synchronous fluorescence corresponding to NDPhA (3×10^{-8} mol L^{-1}), DPhA (9×10^{-8} mol L^{-1}) and a mixture of both at the same concentration.

The synchronous fluorescence spectra were collected by scanning the excitation wavelength between 210 and 350 nm in the wavelength interval 20–160 nm (at λ increments of 5 nm), and were displayed as contour plots (Fig. 6a and b). As can be seen, use of a single scan for determination of mixture causes that fluorescence intensity of compounds decrease compared with that of their individual solution and this approach could not be used to sensitively resolve the mixture. Whereas, by using two different scan modes in addition to the good resolution, there is no loss of sensitivity. The best resolution of the mixture is obtained when the $\Delta\lambda$ of 127 nm for NDPhA and that of 75 nm for DPhA were selected (Fig. 6c). These values are in conformity with those which could be obtained from emission and excitation normal spectra (Fig. 1).

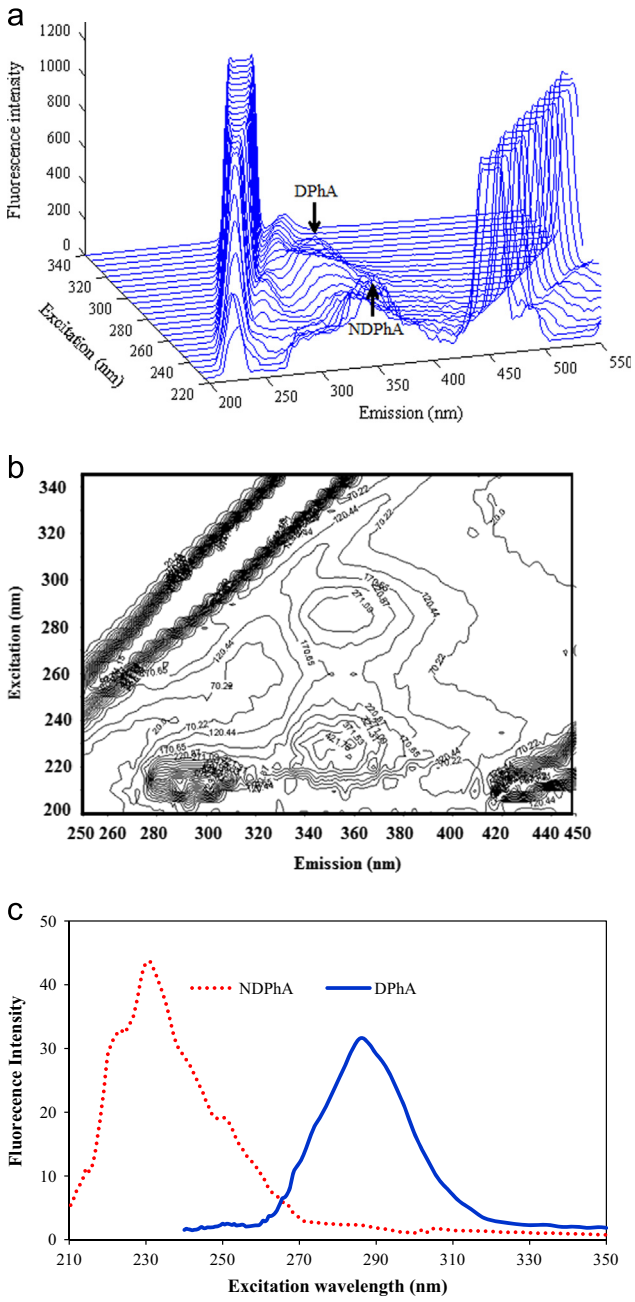

Fig. 5. Three-dimensions fluorescence spectra (a), contour plot (b) of the NDPhA (3×10^{-8} mol L^{-1}) and DPhA (9×10^{-8} mol L^{-1}) mixture and synchronous fluorescence with double scans spectra of NDPhA, dash line (3×10^{-8} mol L^{-1}) and DPhA, solid line (5×10^{-8} mol L^{-1}) in water; $\Delta\lambda=130$ nm for NDPhA and $\Delta\lambda=75$ nm for DPhA (c).

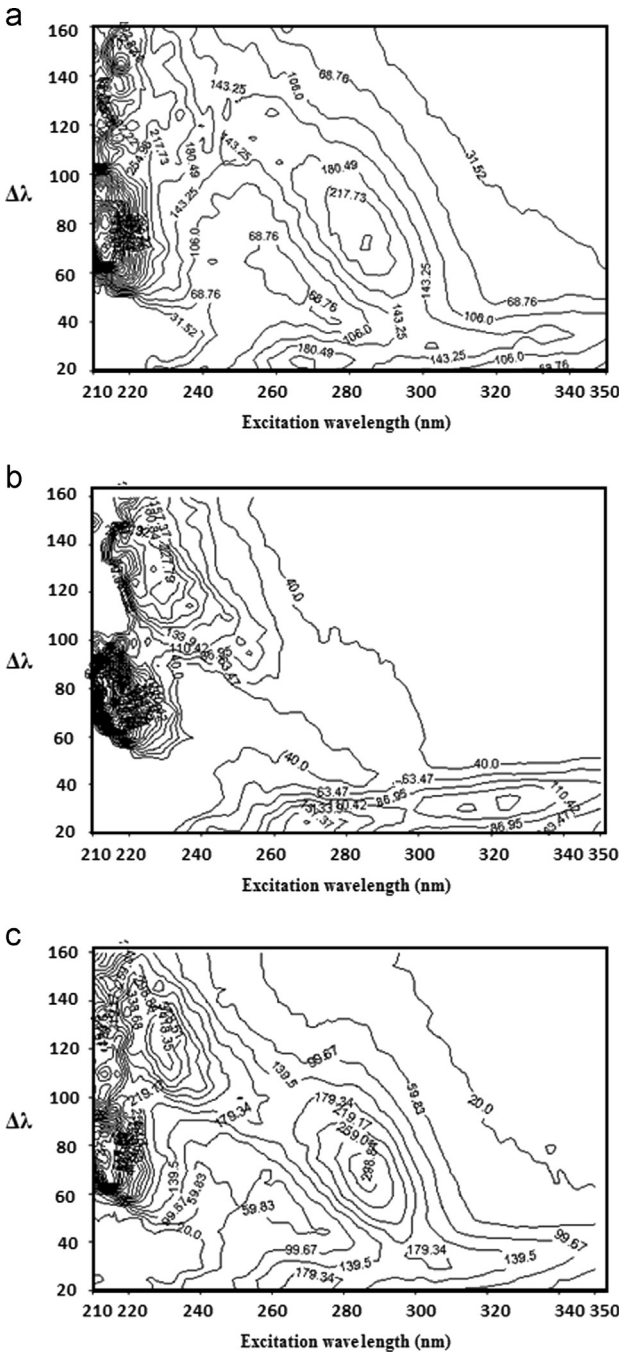

Fig. 6. Contour plots of 9×10^{-8} mol L^{-1} of DPhA (a), 3×10^{-8} mol L^{-1} of NDhPA (b) and mixture of NDhPA and DPhA at the same concentration (c) with the different wavelength interval ($\Delta\lambda$).

The solvent is one of the factors which most influences on the fluorescence signal. Based on our previous work [32] and the results are listed in (Section 3.2), it has been found that water and methanol produced the very good synchronous fluorescence intensities for the mixture of NDPhA and DPhA. Thus methanol and water can be chosen as the diluting solvents throughout the determination of NDPhA and DPhA.

3.5. Analytical figures of merit

The LOD was obtained as the sample concentration which causes a peak that is three times as high as the baseline noise level. Under optimum of condition, results showed that there was a linear relationship between the fluorescence intensity and the concentration in the range of 1×10^{-8} to 6×10^{-6} mol L^{-1} for NDPhA and 4×10^{-8} to 9×10^{-6} mol L^{-1} for DPhA (in water) and 6×10^{-8} to 1×10^{-6} mol L^{-1} for NDPhA and 2×10^{-8} to 5×10^{-6} mol L^{-1} for DPhA (in methanol). The correlation coefficient for the standard calibration graphs were 0.996 and 0.997 (in water, $n=10$), 0.998 and 0.995 (in methanol, $n=10$) for NDPhA and DPhA, respectively. The detection limits were 8×10^{-9} mol L^{-1} for NDPhA and 1×10^{-8} mol L^{-1} for DPhA (in water) and were 2×10^{-8} mol L^{-1} for NDPhA and 1×10^{-8} mol L^{-1} for DPhA (in methanol).

The influences of foreign coexisting substances such as 2-nitrodiphenylamine, 4-nitrodiphenylamine and 2,4-dinitrodiphenylamine

Table 1
Application of the Synchronous fluorimetry method for determination of the DPhA and NDPhA in their synthetic mixtures.

Sample no.	NDPhA			DPhA		
	Added ($\times 10^{-7}$ mol L^{-1})	Found ($\times 10^{-7}$ mol L^{-1})	Recovery (%)	Added ($\times 10^{-7}$ mol L^{-1})	Found ($\times 10^{-7}$ mol L^{-1})	Recovery (%)
1	5.40	5.54	102.6	7.12	7.39	103.8
2	1.64	1.79	109.1	4.56	4.47	98.2
3	2.29	2.15	93.6	3.34	3.25	97.5
4	5.72	5.70	99.8	4.74	4.87	102.7
5	4.75	4.86	102.3	8.84	9.23	104.4

Table 2
SF-DS determination of NDPhA and DPhA in well water sample.

Sample no.	NDPhA			DPhA		
	Added ($\times 10^{-7}$ mol L^{-1})	Found ($\times 10^{-7}$ mol L^{-1})	Recovery (%)	Added ($\times 10^{-7}$ mol L^{-1})	Found ($\times 10^{-7}$ mol L^{-1})	Recovery (%)
1	0.36	0.34	96.0	6.49	6.64	102.3
2	1.33	1.40	105.2	2.43	2.61	107.2
3	0.45	0.50	109.6	4.34	4.19	96.5
4	1.88	2.01	106.9	1.66	1.63	98.4
5	0.89	0.87	97.6	7.33	7.65	104.4
Average RSD (%)	2.3			2.5		

Table 3
Comparison of results of DPhA and NDPhA analysis of gunpowder samples using SF-DS and HPLC method.

Sample no.	NDPhA				DPhA			
	SF-DS[a] (%)	HPLC (%)	*t*-Value	Recovery (%)	SF-DS (%)	HPLC (%)	*t*-Value	Recovery (%)
1	0.295 ± 0.027	0.284	2.22	103.9	0.380 ± 0.018	0.352	2.37	108.0
2	0.651 ± 0.018	0.668	3.95[c]	97.5	0.709 ± 0.009	0.682	0.91	104.0
3	N.d.[b]	N.d.	–	–	1.616 ± 0.023	1.560	1.08	103.6
4	0.659 ± 0.012	0.668	2.24	98.7	0.364 ± 0.010	0.358	0.94	101.7
5	N.d	N.d.	–	–	1.935 ± 0.004	1.940	0.937	99.7

[a] Synchronous fluorimetry-double scan.
[b] Not detected.
[c] Statistically significant at 0.05 level and insignificant at the level of 0.01; The Critical *t*-value for two tail distribution student paired *t*-test having degree of freedom 5 and level of confidence of 95% is 2.57 and for level of confidence of 99% is 4.30.

were tested. It can be found, none of the compounds do not show interference in irradiation and determination steps and could exist in at least 100-fold more than the concentration of NDPhA and DPhA. The proposed method was applied to the simultaneous determination of NDPhA and DPhA in synthetic mixtures containing different concentrations of both molecules. The relative synchronous fluorescence intensities were used and the concentrations of targets in the synthetic mixtures were calculated according to the linear regression equation of the calibration graphs. The results indicate high accuracy of the proposed method as shown in Table 1.

3.6. Analytical application to real samples

The applicability of synchronous fluorometry using double scans (SF-DS) method to the real samples was investigated by determination of NDPhA and DPhA in deferent samples. The well water sample (water was taken from Tehran) selected as an environmental sample. SF-DS of non-spiked samples provided a spectrum without any peaks which is related to NDPhA and DPhA. Thus, the samples were spiked with NDPhA and DPhA at different level, and five replicate analyses were performed for each sample using PIF method followed by SF-DS at optimal conditions. Results obtained by the proposed method and amounts added are in satisfactory agreement. The analytical results are summarized in Table 2. Also, the reliability of the proposed method to the solid samples was investigated for the gunpowder samples. Detection limits of the method are well below levels ($< 2 \times 10^{-8}$ mol L^{-1}), allowing a dilution of the samples. Experimental conditions for these measurements were similar to those described in experimental section. Repeatability studies were satisfactory, giving RSD% values of 1.5 and 2.1 for determination of DPhA and NDPhA, respectively; when reproducibility studies were undertaken over the two sets of five standards for each compound on consecutive days no significant differences were found between the two sets of five replicates at a confidence level of 95%. The results of determination of in DPhA and NDPhA gunpowder samples were compared with the HPLC method which has been performed in separated laboratories. The Student's *t*-test indicates that the differences between the predicted values of concentrations are not significant (Table 3).

4. Conclusion

A new simple and sensitive synchronous fluorescence method combined with PIF for determination of NDPhA and DPhA in gunpowder and well water samples has been developed. One of

Table 4
Comparison of the proposed method with other analytical techniques for determination of DPhA and NDPhA in different samples.

Analytical technique	Description	Sample	DLR[a]	LOD[b]	Analysis time (min)	Recovery (%)	RSD[c] (%)	Ref.
HPLC/TS/MS	The method is based on the application of thermospray HPLC/MS to distinguish NDPhA and DPhA in a highly contaminated soil from an abandoned hazardous waste site	Soil sample	–	~50–100 ng	~35	99–114	–	[15]
LC–ESIMS	A gradient reversed-phase LC method was modified to facilitate detection ESIMS in the positive ion mode	Smokeless powders	0.05–20 mg mL^{-1}	–	> 20	–	< 8.4 [d]	[36]
LC-TEA	A liquid chromatography (LC) coupled with a thermal energy analyser (TEA)	Diphenylamine formulations	0.7–4.1 μg mL^{-1}	0.2 μg mL^{-1} (2 × S/N)	> 10	86–137% (average recovery 103%)	–	[37]
Tandem MS	Tandem MS in the mode of multiple reaction monitoring (MRM) was used for determination of DPhA and its four derivatives	Smokeless gunpowder	5.0–200.0 ng mL^{-1} for DPhA 2.0–100.0 ng mL^{-1} for NDPhA	1.0 ng $mL^{-1}$for DPhA0.5 ng mL^{-1} for DPhA	< 5	80.3 for DPhA79.6 for DPhA	–	[38]
CEC–MS	Determination was performed using capillary electrochromatography (CEC) coupled to time of flight-mass spectrometry (TOF-MS) methods	Smokeless powder	–	0.6 μg mL^{-1}	> 20	–	< 14	[39]
DPP	Differential pulse polarography (DPP) was applied to The simultaneous determination of DPhA drivatives	Simple base solid propellant	–	≥ 0.024 μg mL^{-1}	–	–	–	[40]
SF-DS[e]	Synchronous fluorescence method combined with photochemically induced fluorescence	Liquid and solid samples (well water and smokeless gunpowder)	2–1200 ng mL^{-1} for NDPhA 6.8–1500 ng mL^{-1} for DPhA	1.6 ng mL^{-1} 1.7 ng mL^{-1}	5	98–108	< 3	This work

[a] Dynamic linear range.
[b] Limit of detection.
[c] Relative standard deviation.
[d] Intra-assay precision.
[e] Synchronous fluorimetry-double scan.

the components of the mixture is monitored by measuring its native fluorescence and the other after its UV irradiation and generation of a strongly fluorescent photoproduct. The performances of the PIF method are compared to literature data obtained with other techniques. The proposed method is not time consuming, do not involve any sample cleanups and derivatization. This method has been proved a very useful technique for simultaneous analyzing NDPhA and DPhA in mixture. The comparison of the main characteristics of SF-DS method for determination of NDPhA and DPhA with other reports in literature is summarized in Table 4. The proposed method was found to be easier than the published chromatography and Mass methods, also less time consuming compared with other published LC methods for the simultaneous determination of NDPhA and DPhA, whereas there is no need for using internal standard, gradient elution, or time programming to adjust excitation and emission wavelengths. The technique offers good performance in terms of precision (R.S. D. $< 3\%$), LOD (8×10^{-9} and 1×10^{-8} mol L^{-1} for NDPhA and DPhA, respectively) and is well suited for the quantification of NDPhA and DPhA in real samples without any previous treatment, make this method promising for routine analysis.

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